There also appears to be massive cell death in the somites and notochord of double mutants. We have also examined gene expression using RNA in situ hybridization to look at the effect of the deletion of Foxa1 and Foxa2 on other genes required for notochord formation and NP development. Study of the role of Foxa family action in IVD development may provide insight into new treatments for disk degeneration.

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**Program/Abstract #440**
**Differential requirement of ZIC3 function in cardiac development and X-linked heterotaxy**
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Heterotaxy, contributing to ~5% of congenial heart defects (CHD), arises from abnormal left-right patterning. Mutations of ZIC3 gene (Zinc finger protein of cerebellum 3) are associated with human X-linked heterotaxy. A mouse model with targeted disruption of Zic3 exhibited ~75% early lethality, and recapitulated the phenotype seen in human patients. However, it is not known whether ZIC3 is required in a single developmental field or whether it has pleiotropic roles in multiple developmental processes, and the detailed mechanism remains elusive. To address these questions, we generated a conditional allele of the Zic3 gene by flanking its 1st exon with loxP sites. Sox2-cre, Wnt1-cre and T-cre lines were used to delete Zic3 in epiblast, neural crest and mesoderm, respectively. Deletion of Zic3 in epiblast and mesoderm, but not in neural crest, led to ~50% early lethality. Examination of epiblast conditional embryos by microscopy revealed multiple CNS and neural tube defects similar to the null embryos. But these defects were not found in mesoderm or neural crest conditional embryos, suggesting that Zic3's function in CNS development likely remains intact in these mutants. MRI scanning of Zic3 epiblast and mesoderm conditional embryos also uncovered multiple heterotaxy related visceral abnormalities. Gene expression analysis by microarray in the hearts of embryos at 15.5 dpc revealed a similar expression pattern between Zic3 epiblast conditional and null males, which was significantly different from control males. Perturbed expression of several cardiac genes and direct targets of Zic3 suggested that Notch, BMP and TGF-β signaling might be affected, and requires further investigation.

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**Program/Abstract #441**
**Hox genes control the axis elongation process in chicken embryo**
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The vertebae's precursors, the somites, are formed periodically by the segmentation of the presomitic mesoderm (PSM) which forms by progressive cell deposition from a posterior growth zone. The number of somites is precisely defined for any given species but varies widely from one species to another. In order to maintain a precise number of somites, the body axis elongation has to be tightly controlled. Indeed, using time-lapse imaging of developing chicken embryos we observed that elongation process slows down few hours before the termination of the axis. We previously showed that a gradient of random cell motility within the PSM is implicated in axis elongation (Benazeraf et al., 2010). However the precise control of how the elongation will slow down to define the axis length remains unknown. To address this issue, we used the electroporation technique coupled to time-lapse imaging of developing chicken embryos. Using these techniques we show that cell motility in the PSM decreases progressively at the end of axis elongation. Nevertheless this decrease in cell motility is not sufficient to explain the slowing down of axis elongation. We previously showed that Hox genes are expressed in a collinear fashion in the PSM precursors and control the timing of ingression of the PSM precursors (Iimura and Pourquie 2006). Overexpression of different Hox genes alters body axis elongation. This effect takes place in part by controlling cell motility in the posterior PSM but mainly by regulating the flux of cells ingressing in the PSM. Altogether we propose a new mechanism explaining how the collinear expression of the Hox genes regulates the length of the body axis.

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**Program/Abstract #442**
**Role of 5′HOXD genes in the endochondral ossification**
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Mutations with gain and loss-of-function of Hoxd genes present notably osteogenic defects indicating the involvement of these genes in the endochondral ossification. To clarify the role of Hoxd genes in endochondral ossification, we have analyzed the osteochondrogenic program in the autopod of mice lacking Hoxd11 to 13 (HoxdDel11–13/Del11–13), the animal model for the human synpolydactyly. This mutant is characterized by short and sometimes biphalangeal digits and by an extremely ossification delay. The maximum phenotypic defect occurs in the metacarpals/metatarsals that at birth lack the primary ossification center and collar bone. Ossification center the phalanges is partially abnormal and ventrally biased. During embryonic development Ihh and Runx2 expression is undetectable in the chondrocytes and perichondrium respectively, reflecting the abnormal organization and differentiation of the bone anlagen. The similarity of the phenotype with that of Ihh mutants prompted us to perform the compound Gli3; HoxdDel(11–13) mutant. Interestingly, removal of Gli3 from the HoxdDel(11–13) background rescued ossification in the hindlimb (metatarsals) but only partially in the forelimb (metacarpals). Our results support the involvement of Hoxd11–13 in the formation of the perichondrium and in the regulation of Ihh expression. Supported by grant BFU2008-00397 from the Spanish Ministry of Science and Innovation.

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**Program/Abstract #443**
**HMGB factors are required for posterior digit development through integrating Shh, Wnt and BMP signaling pathways in the forelimb**
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The Hmgb1 and Hmgb2 genes code for chromatin factors, which have critical roles in cellular processes, including transcription and DNA modification. In embryonic development, however, the function of Hmgb genes is largely unknown. To address this issue, we generated double mutants of Hmgb1;Hmgb2 in mice. While double null embryos arrest at E9.5, Hmgb1−/−;Hmgb2+/− embryos exhibit a loss of digit5, the most posterior digit, in the forelimb. We show that Hmgb1−/−;Hmgb2+/− forelimbs have a reduced level of Shh signaling, as well as a significant downregulation of Wnt and BMP target genes in the posterior domain. Furthermore, we demonstrate that hmgb1 and hmgb2 in zebrafish embryos enhance Wnt signaling in a variety of tissues, and that double knockdown embryos have reduced Wnt signaling and shh expression in pectoral fin buds. Our data show that Hmgb1 and Hmgb2 function redundantly to enhance Wnt signaling in embryos, and further suggest that the development of digit5 is regulated by integration of Wnt, Shh and BMP signaling in forelimbs.

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**Program/Abstract # 444**
**Total loss of limb bud retinoic acid signaling in Rdh10 mutants does not affect limb patterning but results in interdigital webbing**

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Expression of genes controlling proximodistal (Meis2) or anteroposterior (Shh) limb patterning is purported to be controlled by retinoic acid (RA). Embryos lacking RDH10, the primary enzyme synthesizing retinaldehyde (needed for RA synthesis) during mouse development, survive until E14.5 with stunted forelimbs but apparently normal hindlimbs. Using embryos carrying the RARE-lacZ RA-reporter transgene, we show that endogenous RA activity in Rdh10 (trex/trex) mutants is detected in neuroectoderm but not limbs during initiation and patterning. Treatment of Rdh10 mutants with 25 nM RA restores RARE-lacZ activity to limb mesoderm, validating that RARE-lacZ is a sensitive marker of RA activity and verifying that RA is absent in mutant limbs. In Rdh10 mutants, hindlimbs exhibit normal Meis2/Shh expression and skeletal patterning via alcian blue staining. Forelimbs also exhibit normal Meis2 expression and only a minor alteration in Shh expression, which may be due to stunted forelimb growth, suggesting a forelimb specific initiation requirement rather than a role in patterning. Rdh10 mutants lack interdigital RA activity later in development, and accordingly fail to exhibit normal loss of interdigital mesenchyme. These findings show that Rdh10 is the sole provider of retinaldehyde needed to generate RA for the limb buds. Our analysis of Rdh10 mutants demonstrates that RA is unnecessary for control of limb patterning but required later for interdigital tissue loss.

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**Program/Abstract #445**
**Nucleo-cytoplasmic shuttling of Tbx5 affects migration of limb precursor cells**

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Recent work from our laboratory demonstrated tbx5 transcriptional regulation by dynamic shuttling of Tbx5 between the nucleus and cytoplasm and retention of the transcription factor along the actin cytoskeleton via the PDZ-LIM protein, Pdlim7. In the zebrafish, tbx5 and pdlim7 are coexpressed in the lateral plate mesoderm, a tissue layer containing both heart and limb precursor cells. Misregulation of Tbx5 via Pdlim7 knockdown causes heart valve malformations and limb bud outgrowth problems, suggesting a basic cellular function for Tbx5/Pdlim7 interactions in the heart and limb cells. For productive limb outgrowth, Fgf signaling between the mesenchyme and the apical ectodermal ridge (AER) is essential. Blocking Pdlim7 expression prevents the switch of fgf24 from the mesenchyme to the AER, resulting in breakdown of the Fgf signaling feedback loop and precluding limb outgrowth. Prior to this signaling failure, we see protracted migration of a population of the common heart-limb field cells (hand2 positive) and reduced cell compaction at the limb fields. Knockdown of Tbx5 has been reported to cause limb cell migration defects. Considering our new findings with Pdlim7, we hypothesize that Pdlim7-mediated Tbx5 shuttling is a regulatory mechanism for cell migration. To explore this, we are currently manipulating the cellular level and subcellular location of Tbx5. To this end, we employ knockdown or overexpression of Pdlim7 in conjunction with wild type and tbx5 mutant (heartstrings) zebrafish crossed to a hand2:eGFP reporter line. Using confocal microscopy, we track the migration behavior of limb precursor cells within the living embryo as a function of Tbx5 and Pdlim7 status.

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**Withdrawn**

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**Program/Abstract #447**
**Prolonged FGF signaling is necessary for foregut organ induction in *Xenopus***

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The FGF signaling pathway plays many roles in patterning the developing gut tube. During organ induction, different thresholds of FGF activity induce the lung, liver, and pancreas lineages in the mouse. The mechanism that regulates the dose of FGF in vivo is unknown. We hypothesize that prolonged FGF signaling is required and that this duration is important to help achieve the thresholds of FGF necessary for proper foregut organ induction. We tested the temporal requirement of FGF signaling by treating developing *Xenopus* embryos with soluble FGF inhibitor molecules over a time course prior to organ induction. Additionally, we injected a dominant negative FGF receptor to block FGF signaling in the endoderm. Embryos were analyzed for markers of the lung, liver, pancreas, and heart to determine if organ